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(21) International Application Number: PCT/GB99/02108 (22) International Filing Date: 2 July 1999 (02.07.99) (30) Priority Data: 9814259.9 2 July 1998 (02.07.98) GB (71) Applicant (for all designated States except US): UNIVERSITY OF HERTFORDSHIRE [GB/GB]; Hatfield, Hertfordshire AL10 9AB (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): TRACEY, Mark, Christopher [GB/GB]; 5 The Old School, Mount Pleasant, Hertford Heath, Hertfordshire SG13 7QX (GB). (74) Agent: WHITAKER, Iain, Mark; Sommerville & Rushton, 45 Grosvenor Road, St Albans, Hertfordshire AL1 3AW (GB).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	

(54) Title: FABRICATION AND METHOD OF USE OF CODED PARTICLES IN THE FIELD OF COMBINATORIAL CHEMISTRY

(57) Abstract

A method for screening to identify a compound of interest from a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available in a database to identify the sequence of reactions experienced by substantially each support particle, the method comprising the steps of: (a) dividing the compound library into two sets there being a statistically satisfactory representation of all compounds in the library within each set; (b) sub-dividing a first one of the two sets into a number of sub-sets; (c) testing for a chemical activity of interest in any one of the said sub-sets and identifying the one or more sub-sets showing the activity of interest and reading the machine readable codes of the support particles in that or those sub-set(s); (d) checking a database of the tracking data, or data established from the tracking data, to identify machine readable codes of any other support particles which have been through the same steps and therefore, have the same chemical structure of compound on the support particle; and (e) reading the machine readable codes of the support particles in the second set of particles to locate the one or more support particles having the corresponding machine readable code(s) and retrieving that or those support particles and subjecting it or them to further screening tests to identify the compound of interest.

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**FABRICATION AND METHOD OF USE OF CODED PARTICLES IN THE FIELD OF
COMBINATORIAL CHEMISRTY**

Field of the Invention

- 5 The present invention relates to coded Combinatorial Chemistry particles. It is particularly applicable to improved methodology for reading and using the coded particles.

Background to the Invention

- 10 A method of fabricating and using coded particles in the area of combinatorial chemistry library synthesis is described in GB 2306484 B which describes the fabrication of coded particles of dimensions typically in the order of 100 μ m length and width and typically in the order of 20 μ m thickness. Additionally, the particles are encapsulated by, coated with, or otherwise attached to, a similar volume of
- 15 polymeric resin material which acts as a substrate for the chemical compound growth during the combinatorial chemistry process. Each particle carries a unique code, thus allowing individual particles to be tracked throughout the combinatorial compound library synthesis, an essential requirement in allowing the compound synthesis sequence for compounds which exhibit the desired biological,
- 20 pharmacological or chemical activity to be determined and reproduced. A more detailed description of such a synthesis is given in GB 2306484 B, the entire contents of which are hereby imported by reference. It is intended that this earlier disclosure should form an integral part of this present application.

this layer by etching away the revealed areas of the layer between the particle outlines and within the areas designated as code marks. The particles may then be freed from the wafer substrate by dissolution of the sacrificial layer which underlies the particles. The sacrificial layer is typically
5 made of aluminium if the particles are of silicon dioxide, and of silicon dioxide if the particles are of silicon.

In order to render the particles suitable for use in combinatorial chemistry, an additional processing stage is required before the particles are freed from the wafer
10 substrate. In this additional stage, each coded particle receives its quantity of polymer support material attached to the particle in the form of a chemically attached surface layer, by mechanical linkage, or by some other method of attachment as described in GB 2306484 B. The microscopic code on the particles may be interrogated and read using contemporary microscope-based image
15 processing systems. By way of example, a code containing just twenty binary sites (pits, holes, or similar features) would allow a million particles to be uniquely numbered from 1 to 1,000,000.

The accepted method of manufacture of the coded particles as described in GB
20 2306484 B is limited in the maximum thickness which the particles can assume. Because the particles are created from a deposited layer, this maximum thickness corresponds to the layer depth above which material stresses can result in particle fracture when the particles are freed from the host substrate wafer. Typically this thickness is of the order of 10-20 micrometres. In some areas of combinatorial
25 chemistry, it is valuable to produce more compound per particle than may be supported by a particle of this size. However, if the particle length and width are

(e) reading the machine readable codes of the support particles in the second set of particles to locate the one or more support particles having the corresponding machine readable code(s) and retrieving that or those support particles and subjecting it or them to further screening tests to identify the compound of interest.

5 Preferably, in the method prior to step (b) the compound bound to each support particle is released by relieving the compound linking it to the support particle to release each compound into a liquid medium.

In step (c) prior to reading the machine readable codes of the support particles they are preferably removed from a vessel in which they were tested for
10 chemical activity and transferred to a reading station.

Advantageously the reading of the codes on the coded particles is carried out by firstly placing the particle to be read in a substantially flat, substantially horizontal vessel with a transparent bottom, the area of the bottom being at least double the total area of the particles to be measured; agitating the vessel to form a
15 monolayer of particles on the bottom; and scanning the vessel with an imaging system whilst illuminating the particles.

Preferably the reading station is adapted to automatically read the machine readable codes of the support particles and to automatically identify the one or more support particles with machine readable codes in step (e) that correspond to the
20 codes from the database identified in step (d).

Suitably the one or more corresponding support particles are automatically retrieved or pinpointed by a laser beam, or other means, from the second set of particles.

According to a second aspect of the present invention there is provided a
25 system for use in the method of any preceding claim and which comprises at least two primary vessels each containing a respective one of the two sets of support

- (b) suspending the support particles in a fluid;
- (c) dividing the fluid containing the particles into a plurality of portions, reading and recording the machine readable codes during or after the division process in order to track the movement of specific particles into respective portions;
- 5 (d) subjecting respective portions to specific chemical reactions;
- (e) recombining the respective portions; and
- (f) repeating steps (c), (d) and (e) as necessary so as to create a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available to identify the
- 10 sequence of reactions experienced by substantially each support particle, and wherein following a division of the particles the codes are read and analysed to determine whether any combinatorial permutation is significantly under represented.
- Suitably any under-representation of any permutation of chemical combination is adjusted for by re-mixing all or some of the sub sets of particles and,
- 15 optionally, re-reading the machine readable codes.

Description of the Drawings

The present invention will now be described, by way of example only, with reference

20 to the accompanying drawings wherein:-

Figures 1A and 1B illustrate in perspective view coded particles onto which one and two polymer deposits respectively have been made;

Figure 2 illustrates plan and side views of a resin bead trapped within an aperture in a coded particle.

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Description of Preferred Embodiments

Compart Technology Ltd, Peterborough, UK). The double-sided polished silicon wafer is first anodic-bonded to a substrate material such of glass or by other means to other rigid material such as an organic solid. In the preferred embodiment a Pyrex glass wafer is used.

5

However, any suitable substrate may be used which has sufficient mechanical strength and chemical stability to withstand the rest of the process. This includes certain rigid polymers and wax blocks, such materials being selected by the materials specialist.

10

The upper surface of the silicon wafer is then coated with an appropriate photoresist material which is subsequently exposed to ultraviolet radiation through a photolithographic mask which defines the size and shape of the particles and their respective code marks. The particle size thus defined will be selected in accord with the wafer thickness, such that the length and width dimensions of the particle are preferably several times the particle thickness so as to ensure a high probability that a free particle will lie horizontally, thus facilitating code reading. For a 300 micrometre thick wafer therefore, particles of the order of 700 micrometres square would be appropriate. Thinner wafers would allow commensurately smaller cross-sectional dimensions. Unexposed areas of photoresist are removed by an appropriate solvent to expose unwanted areas of the silicon surface. These unwanted areas are the inter-particle material plus the sites in the particle defining the code marks. A plasma etch process may then be used to etch the exposed material of the silicon wafer through the entire wafer thickness but without any etching of the underlying bonded glass wafer: the etching stops at this interface. Both the particles and their code 'holes' are thus formed in the same operation.

may also be advantageously pre-treated with a silane compound designed to provide a 'coupling' functionality between the silicon dioxide and the polymer, hence promoting good adhesion. Also, the area of the particle surface to which polymer is to be applied may itself be etched by appropriate masking and plasma etching to
5 leave a well or wells into which the polymer is forced, again promoting good adhesion between the particle and the polymer.

The use of a silk-screen mask in this way allows accurate simultaneous deposition of polymer to each particle on the wafer, possibly many thousands of particles in
10 total. As indicated in Figure 1, each particle 1 would thus exhibit a code in the form of holes 2, and an area or several separate areas of polymer 3. The exact positions of code holes and polymer would be such as to ensure unobscured viewing of the code holes. Once the polymer has been deposited in this way, the temperature of the whole wafer assembly may be raised to an appropriate level to ensure cross-
15 linking of the polymer takes place, whereupon the bond between the polymer and the particle becomes permanent. Finally the resulting polymer-patterned glass silicon composite wafer may be immersed in hydrofluoric acid in order to dissolve ('sacrifice') the glass substrate, thereby releasing the discrete silicon particles into the acid. The acid may be subsequently removed and the particles recovered for
20 use.

In some cases the use of an encapsulating layer (for instance a wax-like compound) resistant to HF to protect the upper surface of the silicon-glass composite subsequent to polymer application and curing would be advantageous. This facility
25 would serve both to protect the aforementioned silane and additionally provide for the use of a polymer other than polystyrene which may be desirable for chemical

The size of the entrapment hole is therefore chosen to be fractionally less than the smallest size which the bead is likely to assume during processing. This would normally occur when the bead is immersed in diethyl ether or similar reagent. The polymer beads may be linked to the silicon particles in the following way. When the silicon particles are etched but still attached to the glass substrate, the suitable population of resin beads is prepared by immersion in diethyl ether, causing them to contract to minimum dimensions. The collection of beads is then poured over the wafer surface and a roller or similar device used to compress the beads against the silicon particles. In areas where a bead becomes positioned over an entrapment hole, the bead will be forced into the hole. Repeated cycles of this process will ensure that the vast majority of the particles entrap a bead. Loose particles may then be flushed away and the wafer immersed in hydrofluoric acid as before to release the particles from the glass substrate. Each silicon particle will then contain a polymer resin bead exposed on upper and lower surfaces, allowing expansion of the bead without it being dislodged from the host silicon particle.

It is intended that this disclosure should encompass particles made from material other than silicon. Any suitable material can be used providing it will undergo the process steps described or their technical equivalent. For example, metals such as gold or compounds such as silicon dioxide could be substituted for silicon.

The invention also includes other methods of trapping a polymer resin bead such as those described in GB 9803182.6.

Reading the Codes

In the original patent (GB 2306484 B), the coded particles therein described were

Illumination of the particles can be from below, in which case the code holes appear as bright spots against a dark background, or from above, in which case (because of the surface reflectivity of the silicon) the code holes appear as dark spots against a light background. Both forms of illumination can be used sequentially to ensure optimal imaging of the particles and hence greatest accuracy in reading the codes. The process of reading the code information from the recorded images of particles is as described in GB 2306484 B. It would also be possible to configure the mechanically driven stage such that the vessel is vibrated rapidly. This may be useful to ensure at the start of the scanning process that any particles which rested on their side would topple to the more stable flat orientation required for code reading. In the normal course of events, however, the particles are considered to be static within the vessel such that the images of the particles can be used to not only determine the codes on individual particles but also, in conjunction with a knowledge of the stage motion, the position of each particle can be evaluated. Knowledge of the position of each particle at and after the final code reading process can be of value because it allows the user to either manually or robotically find and extract a particle or particles of choice. Examples of the use of this facility are given below.

Improvements to Combinatorial Processing

The following improvements to the actual methodologies of combinatorial processing are realisable because of the unique ability of the coded silicon particles to allow particle tracking of all particles throughout the split-and-pool process steps by reading codes at each 'split' stage. This is in contrast to conventional 'chemical tagging' of the particles (polymer beads) which allows the process paths of individual beads to be determined only at the termination of the complete compound synthesis process.

the vessel showing the activity and placed individually into separate vessels (one bead per vessel) whereupon the remaining half of the compound may be cleaved from the bead. These individual compound solutions are then assayed to identify the active compound. By this means the screening of the population of beads involves
5 (M/N)+N assays rather than M, a significant reduction.

Using the coded silicon particles described in this invention we can achieve the same effect by taking two populations of coded particles through identical process steps or by dividing a larger collection of beads into two approximately equal sets at
10 the end of the synthesis process. In either case two distinct sets of beads are extant: A, B, resulting from synthesis with sufficient redundancy to ensure a statistically satisfactory representation of all chemical structures within each set. One set, for instance A, is reserved for later use and the second set B, is divided at random into M subsets of N beads. The exact value of N being determined by
15 statistical considerations relating to the particular synthesis specification. Small variations about N from subset to subset are not detrimental to the methodology as described here. The synthesised compound bound to the individual beads is preferably released by cleaving the linking compound thus releasing the synthesised compounds into the liquid phase (however options to screen with the compound
20 uncleaved are also applicable to this technique) within the analysis vessel containing the N beads. The coded beads are left in-situ within the vessel.

Established screening procedures, advantageously those employed in High Throughout Screening (HTS), are then employed to test for desirable chemical activity in any of the M subsets and hence vessels. Detection of desirable chemical
25 activity by means (for instance) of fluorescence within on of the M vessels indicates that one or more of the N chemical structures it contains may be responsible.

In this invention we can exploit a synthesis database that connects every chemical structure to a unique code thereby enabling the chemist's set or list of desired compounds to be translated to a list of corresponding head codes from which the automated machine vision particle-code reading system can retrieve the appropriate beads. This identification may be on the basis of beads previously determined spatial co-ordinates or by direct searching. Retrieval may be manual, for instance guided by an optical designating spot (for instance a laser) or it may be fully automated by established robotic techniques. An alternative to the specification of a region of space or a list of compounds would be to "sample" chemical space whereby a certain degree of synthesis path variation is specified and only structures exceeding this difference are extracted. By this means a smaller initial screening library may be extracted that indicates broad trends in activity within the library. In either example the retrieved compounds would be cleaved from the supporting bead into liquid phase for subsequent analysis or may be subjected to screening procedures as previously described.

reading the machine readable codes of the support particles they are removed from a vessel in which they were tested for chemical activity and transferred to a reading station.

5 4. The method as claimed in claim 3, wherein the reading of the codes on the coded particles is carried out by firstly placing the particle to be read in a substantially flat, substantially horizontal vessel with a transparent bottom, the area of the bottom being at least double the total area of the particles to be measured; agitating the vessel to form a monolayer of particles on the bottom; and scanning
10 the vessel with an imaging system whilst illuminating the particles.

5 A method as claimed in any preceding claim, wherein the reading station is adapted to automatically read the machine readable codes of the support particles and to automatically identify the one or more support particles with machine
15 readable codes in step (e) that correspond to the codes from the database identified in step (d).

6 The method as claimed in claim 5, wherein the one or more corresponding support particles are automatically retrieved or pinpointed by a laser beam, or other
20 means, from the second set of particles.

7 A system for use in the method of any preceding claim and which comprises at least two primary vessels each containing a respective one of the two sets of support particles, a reading station to read the machine readable codes of the
25 support particles; a database of the machine readable codes and tracking data; and means programmed to identify the machine readable codes of the corresponding

- (c) dividing the fluid containing the particles into a plurality of portions, reading and recording the machine readable codes during or after the division process in order to track the movement of specific particles into respective portions;
- (d) subjecting respective portions to specific chemical reactions;
- 5 (e) recombining the respective portions; and
- (f) repeating steps (c), (d) and (e) as necessary so as to create a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available to identify the sequence of reactions experienced by substantially each support particle, and
- 10 wherein following a division of the particles the codes are read and analysed to determine whether any combinatorial permutation is significantly under represented.
- 12 A method as claimed in claim 10, wherein any under-representation of any permutation of chemical combination is adjusted for by re-mixing all or some of the
- 15 sub sets of particles and, optionally, re-reading the machine readable codes.

1	International Application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01J19/00 G01N33/58 G01N33/543

B. FIELDS SEARCHED

IPC 7 B01J G01N

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	<p>WO 97 19958 A (WLODEK MANDECKI) 5 June 1997 (1997-06-05) abstract page 1, paragraph 1 page 2, line 19 - page 3, line 8 page 9, line 7 - line 32 page 10, line 6 - line 31</p> <p style="text-align: center;">—</p> <p style="text-align: center;">-/-</p> <p style="text-align: center;">.</p>	<p>9</p> <p>1-8, 10-12</p>

☒ Patent family members are listed in annex.

"&" document member of the same patent family

Stevnsborg, N

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/02108

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